PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 21078WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09145	International filing date *(day/month/year)* 15.08.2003	Priority date *(day/month/year)* 19.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/00		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.03.2004	01.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Mundel, C Telephone No. +49 89 2399-7314

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09145

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-59 as originally filed

Sequence listings part of the description, Pages

1-71 as originally filed

Claims, Numbers

1-24 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-24 (partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-24 (partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8, 20, 21
	No:	Claims	1-7, 9-19 and 22-24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

An objection of lack of unity has been raised against the claims of the present application during search. Since the applicant chose to pay no additional search fees, the invention has only be searched as far as it refers to the invention 1 (see International Search Report). Thus, the claims of the present application will only be examined as far as they refer to invention 1.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to Aspergillus niger NBE028 polypeptide (SEQ ID NO:3) encoding a lipolytic enzyme and to the corresponding polynucleotides (SEQ ID NOs: 1 and 2), to vectors comprising said polynucleotides, to host cells comprising said polynucleotides or vectors, to antibodies directed against the NBE028 polypeptide and to the use of the NBE028 polypeptide in the preparation of a dough or baked product.

2. Reference is made to the following documents :

   D1: WO 01/27251 A (NOVOZYMES AS) 19 April 2001 (2001-04-19)
   D2: SUGIHARA A ET AL: 'PURIFICATION AND CHARACTERIZATION OF ASPERGILLUS-NIGER LIPASE' AGRICULTURAL AND BIOLOGICAL CHEMISTRY, vol. 52, no. 6, 1988, pages 1591-1592.
   D3: NAMBOODIRI V M ET AL: 'Purification and biochemical characterization of a novel thermostable lipase from Aspergillus niger.' LIPIDS. UNITED STATES MAY 2000, vol. 35, no. 5, May 2000 (2000-05), pages 495-502.
   D4: TOROSSIAN K ET AL: 'PURIFICATION AND CHARACTERIZATION OF AN ACID-RESISTANT TRIACYLGLYCEROL LIPASE FROM ASPERGILLUS-NIGER' BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, vol. 13, no. 2, 1991, pages 205-211.
   D5: DATABASE GENESEQ [Online] 1 June 2003 (2003-06-01) BRITO A.G. ET AL.: 'Apergillus nidulans triacylglycerol lipase (lipA) gene, wild type allele' retrieved from EBI, accession no. Q876R3.
   D6: DATABASE EMBL [Online] Aspergillus niger EST., 20 September 2000

(2000-09-20) TSANG A. AND STORMS R.: 'Aspergillus niger Expressed Sequence Tags' retrieved from EBI, accession no. BE760259.

D7: WO 00/56762 A (NOVONORDISK AS ;NOVO NORDISK BIOTECH INC (US)) 28 September 2000 (2000-09-28)

D8: DATABASE EMBL [Online] A. nidulans lipA gene, 30 November 2000 (2000-11-30) BRITO A.G. ET AL.: 'Aspergillus nidulans lipase (lipA) gene, where mutation confers resistance to undecanoic acid' retrieved from EBI, accession no. AF315651.

D9: EP-A-0 687 734 (SOLVAY) 20 December 1995 (1995-12-20)

D10: DATABASE UNIPROT [Online] Lipase 2 precursor (EC 3.1.1.3), Candida rugosa, 1 October 1993 (1993-10-01) retrieved from EBI, accession no. P32946.

D11: DATABASE UNIPROT [Online] Lipase 5 precursor (EC 3.1.1.3), Candida rugosa, 1 October 1993 (1993-10-01) retrieved from EBI, accession no. P32949.

3. Novelty; article 33(2) PCT.

3.1 The document D7 discloses an Aspergillus niger EST having 98,8 % identity with SEQ ID NO:1 of the present application in 167 bp overlap (SEQ ID NO: 4074 of D7). This EST will hybridize with SEQ ID NO:1 even under stringent hybridization conditions. Moreover, said polynucleotide has been cloned in a vector which has been used to transform a host cell.

Therefore, the subject-matter of claims 1-4, 9, 12 and 18 cannot be considered as novel over the teaching of D7 (article 33(2) PCT).

The same reasoning applies to D6 which discloses an A. niger EST having 89,9 % identity in 148 bp overlap with SEQ ID NO:1 of the present application. Therefore, the subject-matter of claims 1-4, 9, 12 and 18 cannot be considered as novel in the light of D6 (article 33(2) PCT):

3.2 The documents D8 and D9 (SEQ ID NO:7) disclose nucleic acid sequences having some identity with the sequences disclosed in SEQ ID NO: 1 or 2 of the present application. Said sequences would probably hybridize with the polynucleotides of the present application under non-stringent hybridization conditions. The sequence disclosed in D8 is an A. nidulans sequence and the sequence disclosed in D9 is from A. foetidus. Therefore, and for the

reasons mentioned in point 3.1 above, the subject-matter of claims 1, 3-4, 9, 12 and 18 cannot be considered as novel over the teaching of D8 or D9 (article 33(2) PCT).

3.3 Due to the clarity problem mentioned in point 5.1 below, each polynucleotide encoding a fungal lipase and more particularly an A. niger lipase can be considered to fit the definition of claims 1, 3-4 and 5-7 and each fungal lipolytic enzyme can be considered to fit the definition of claims 13-16. The attention of the applicant is drawn to the fact that numerous A. niger lipases were known in the art (see D1-D4) and that the sequence of A.niger lysophospholipases is disclosed in D1 (SEQ ID NOs: 1-4). D1 discloses the cloning of the polynucleotides encoding the lysophospholipases in vectors and the transformation of cells with said vectors (p. 9, Cloning of llpl-1 gene and p. 12, Cloning of llpl-2 gene). Moreover, D1 discloses the expression of said genes in A. oryzae (p. 9, Expression of llpl-1 gene in Aspergillus oryzae and p. 12, Expression of llpl-2 gene in Aspergillus oryzae) and in A. niger (Example 6). The use of the lysophospholipases in the preparation of dough and bakery product is also disclosed (p. 6, Use of lysophospholipase).

Therefore, the subject-matter of claims 1, 3-4, 5-7, 9-19 and 22-24 cannot be considered as novel in the sense of article 33(2) PCT.

3.4 The subject-matter of claims 8 and 20-21 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, the subject-matter of claims 8, 20 and 21 has to be considered as novel in the sense of article 33(2) PCT.

4. Inventive step; article 33(3) PCT.

As can be seen in documents D1-D4, several Aspergillus niger enzymes having lipolytic activity were well-known and the use of said enzymes in the preparation of dough or bakery products had already been proposed. Said lipases had been purified, their characteristics studied and a N-terminal amino acid sequence disclosed. D1 even discloses the complete nucleic acid and amino acid sequences of two A. niger lysophospholipases.

In the light of these documents, the problem to be solved by the present application can be seen as the provision of a further lipolytic enzyme from A.

niger.

The application solves this problem by the provision of the NBE028 polypeptide (SEQ ID NO:3) and the corresponding nucleic acids (SEQ ID NOs:1 and 2).

The IPEA considers that the mere purification of a further A. niger enzyme having lipolytic activity cannot be considered as inventive since (1) protocols for the purification of such enzymes were well-known (see D2-D4), (2) numerous sequences of fungal lipases were known, (3) the existence of several lipolytic enzymes activities in A. niger was known and the skilled person had not reason to think that all these lipases had been discovered.

Thus, an inventive activity for the provision of the NBE028 polypeptide and corresponding polynucleotides could only be recognized if the selection of said specific polypeptide is motivated by a technical purpose, i.e. a hitherto unknown or unexpected effect due to the specific NBE028 polypeptide over the other well-known A. niger lipases. For the moment, the IPEA fails to see such an effect for the selection of the NBE028 polypeptide. Therefore, the subject-matter of claims 1-19 and 22-24 cannot be considered as inventive in the sense of article 33(3) PCT.

The generation of a fusion protein comprising a non-inventive protein or the generation of antibodies directed against a non-inventive protein cannot be considered as inventive. Therefore, claims 20 and 21 also lack inventive step (article 33(3) PCT).

5. Other remarks about the application.

5.1 In claim 1, no hybridization conditions are given. Therefore, the subject-matter of claim 1 encompasses lots of polynucleotides unrelated with the present application.
Moreover, there is no minimal size given for the polynucleotide of claim what renders the scope of the claim unclear.

5.2 Claim 2 refers to "high stringency hybridization conditions". Due to the numerous, sometimes very different definitions of what "high stringency hybridization conditions" should be in the literature and more specifically in the patent literature, the IPEA considers that the hybridization conditions

should be specified in the claim.

Moreover, there is no minimum size given for the polynucleotide of claim 2 what renders the scope of the claim unclear

5.3 Claim 3 and 4 further characterize the polynucleotides of claims 1 and 2 by the origin of said polynucleotide. The attention of the applicant is drawn to the fact that, once isolated, the only way to determine the origin of a polynucleotide is by reference to its specific sequence.
This remark also applies mutatis mutandis to claim 14.

5.4 In claim 5, reference is made to "functional equivalents" of the polypeptides of the present application. The attention of the applicant is drawn to the fact that any well-known lipolytic enzyme, and more particularly all the well known fungal lipolytic enzymes will fit this definition. So any fungal lipase would deprive claim 5 of novelty.

This remark also applies mutatis mutandis to claims 6 and 13.

5.5 It is not clear which "functional domains" are referred to in claim 6.

5.6 The attention of the applicant is drawn to the fact that each recombinant A. niger cell will fit the definition of claim 18. Recombinant A. niger cells were already known in the art.
This remark also applies to claim 19.

5.7 In claim 22, it is not clear to what the lipolytic enzyme should be added, what renders claim 22 unclear.